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### (54) Process for producing cheese using transglutaminase

Verfahren zur Käseherstellung mit Transglutaminase

Procédé de préparation de fromage avec une transglutaminase

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WO-A-94/21129 WO-A-94/21130

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EP 0 711 504 B1

## Description

Field of the Invention

5 [0001] The present invention relates to a process for producing cheese, and more specifically, it relates to a process for producing cheese using a transglutaminase (hereinafter abbreviated as "TG").

Prior Art and Problems Thereof

10 [0002] Natural cheese (cheese refers to natural cheese hereinafter unless otherwise indicated) was once only scarcely familiar to the Japanese people, but in recent years, various kinds of cheese are obtainable.

[0003] It has been said that there are many kinds of cheese (approximately 400 kinds of cheese) which are usually eaten in foreign countries but are unfamiliar in Japan.

15 [0004] In the production of cheese, it is industrially preferable to form a curd in as large an amount as possible from a fixed amount of starting milk, in view of the production costs and the effective application of milk resources and because products can be distributed to consumers at low costs.

[0005] However, a high yield of curd in the conventional method of producing cheese means, in many cases, that the whey drainage is not satisfactory. As a result, properties of cheese, such as firmness, body, texture and the like are lost, thereby decreasing the quality of the cheese to a low level.

20 [0006] Attempts have been made to improve the yield of the curd by utilizing a whey protein in the production of cheese.

[0007] For example, U.S. Patent No. 4,205,090 describes a method in which milk is concentrated to a volume of approximately 1/3 through ultrafiltration, and cheese is produced using this concentrated milk as a starting material. Japanese unexamined Patent publication of PCT application (Kohyou) No. 501,810/1982 describes a method in which  
25 cheese is produced from a substance, as a starting material, which is obtained by selectively concentrating milk through ultrafiltration, increasing the ion strength of the concentrate, then fermenting the concentrate and removing the water therefrom. Japanese Laid-Open Patent Application (Kokai) No. 308,756/1990 describes that when cheese is produced using a concentrated starting raw milk and a protein of a concentrated whey which is obtained by concentrating the whey formed as a by-product in the production of cheese, the whey protein at a high concentration is contained in the obtained cheese curd, and as a result, the whey protein formed as a by-product can thus be effectively utilized.  
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[0008] However, in these methods, the starting milk or the whey to be reused has to be subjected to a pre-treatment (concentration through ultrafiltration or the like). Accordingly, it is considered that these methods are neither industrially simple, nor is the quality of cheese produced satisfactory enough to the consumers.

[0009] The following documents have already reported milk products using TG. Japanese Laid-Open Patent Application (Kokai) No. 27,471/1989 describes a method of producing cheese which includes a step of adding TG during the production. However, the cheese described in Japanese Laid-Open Patent Application (Kokai) No. 27,471/1989 is produced from a curd which is obtained using glucodeltalactone and TG or TG alone but not a rennet (milk clotting enzyme), and this method is vastly different from the process for producing cheese using the milk-clotting enzyme in the present invention.  
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40 [0010] Japanese Laid-Open Patent Application (Kokai) No. 131,537/1990 involves a method of producing a cheese food using TG. However, the cheese food intended here is produced by heat-melting natural cheese or processed cheese as a starting material. Thus, "cheese food" is classified as a food which is extremely different from natural cheese intended in the process of the present invention.

45 [0011] WO 93/19610 describes a method in which a milk protein solution of which the pH is adjusted in the acidic region by means of a yogurt starter is reacted with TG. However, the above-mentioned invention does not include a step of adding a milk-clotting enzyme, and defines the final product, strictly speaking, as a milk-like product which is different from what the present invention terms cheese.

[0012] WO 94/21129 describes a method of producing an acidic edible gel based on milk and the application of the edible gel produced by this method to cheese. However, the above-mentioned invention does not have any description over addition of a milk clotting enzyme. Accordingly, this method is quite different from the process for producing cheese in the present invention wherein the milk clotting enzyme is added for a reaction. Further, cheese using the edible gel is extremely different from the natural cheese intended in the present invention.

[0013] On the other hand, the following documents report milk products using TG and a milk clotting enzyme.

55 [0014] WO 93/22930 describes a method in which a solution containing a milk protein is reacted with TG to produce a milk-like product. However, there is nothing in the above-mentioned document to describe the production of cheese itself. Further, a method of producing a milk-like product as described in the Examples of this document is vastly different from the process for producing cheese using the milk clotting enzyme in the present invention, and the final product is not cheese itself. Still further, this document does not disclose, at all, the order in which the treatments with TG and

the milk clotting enzyme are to be conducted, that are disclosed in the present invention.

[0015] WO 94/21130 describes a method of producing a non-acidified edible gel based on milk, which comprises reacting a milk protein solution with TG at a first stage, adding a rennet (milk clotting enzyme) to the mixture for a reaction at a second stage, and heat-treating the reaction mixture at a third stage. It also indicates the use of the thus-obtained edible gel to cheese.

[0016] However, the order of the addition of the rennet (milk clotting agent), the addition of TG and the heat treatment is clearly different from the order provided in the present invention.

[0017] WO 93/22930 and WO 94/21130 do not have any description regarding a step for decreasing the pH with the addition of a cheese starter. In this respect as well, the methods disclosed in these documents no doubt differ from the process for producing cheese, namely, natural cheese using a cheese starter in the present invention.

[0018] WO 94/21130 indicates that the rennet (milk clotting enzyme) added does not exhibit ordinary performance (separation of milk into cheese curd and whey) and forms a single-phase gel. Even if such a gel is used in cheese, it is wholly different from cheese which is produced through a step of discharging whey like ordinary natural cheese.

#### Means for Solving the Problems

[0019] The present inventors have assiduously conducted investigations to solve the above-mentioned problems, and have consequently focused on the fact that when TG catalyzes an acyl transfer reaction between a  $\gamma$ -carboxamide group of a glutamine residue and a primary amine in a protein or a peptide chain and furthermore the primary amine is a lysine residue of a protein, an  $\epsilon$ -( $\gamma$ -Glu)-Lys crosslink is formed. As a result, they have found that when steps of adding TG and a milk clotting enzyme for a reaction are incorporated under suitable conditions and in suitable order, the weight of a curd formed is clearly increased, and the obtained curd is transformed into cheese which has a good quality even after it is matured while maintaining the necessary firmness and good body. This finding has led to the completion of the present invention.

[0020] The present invention relates to a process for producing natural cheese, wherein a cheese curd is produced from a solution containing milk or a milk protein and the curd is separated from the whey,

said process comprising the steps of

- (1) adding between 0.1 U and 50 U transglutaminase per gram protein to the solution containing milk or the milk protein for a reaction at a first stage,
- (2) heat-treating the mixture at a second stage to de-activate the transglutaminase and
- (3) adding a milk clotting enzyme at a third stage to react the mixture with the milk clotting enzyme for a fixed period of time.

[0021] A second embodiment of the present invention is directed to a process for producing natural cheese, wherein a cheese curd is produced from a solution containing milk or a milk protein and the curd is separated from the whey,

said process comprising the steps of

- (1) adding a milk clotting enzyme to a solution containing milk or a milk protein at a first stage to react the solution with the enzyme for a fixed period of time, and then
- (2) adding between 0.1 U and 50 U transglutaminase per gram protein to the mixture for a reaction at a second stage.

[0022] A third embodiment of the present invention relates to a process for producing natural cheese, wherein a cheese curd is produced from a solution containing milk or a milk protein and the curd is separated from the whey, which comprises adding between 0.1 U and 50 U transglutaminase per gram protein to the solution containing milk or the milk protein for a reaction and, at the same time, adding a milk clotting enzyme to the solution.

[0023] What the present invention terms cheese refers to natural cheese, and the process for producing cheese includes a step of "acidification" in which a starter is added to starting milk and a step of "renneting" by action of a milk clotting enzyme. There are a great many kinds of cheese. The process of the present invention is directed to all kinds of cheese which are produced by the process including acidification with the starter and the renneting.

[0024] The present invention is characterized in that the step of adding TG for a reaction is incorporated into the production of all kinds of cheese in a suitable order. The step of adding TG for the reaction is incorporated according to the following three processes.

[0025] The first process comprises three stages, that is, adding TG to a solution containing milk or a milk protein at a first stage, heating the mixture at a second stage, and adding a milk clotting enzyme to the reaction mixture at a third stage to react the reaction mixture with the milk clotting enzyme for a fixed period of time.

[0026] The second process comprises adding a milk clotting enzyme to a solution containing milk or a milk protein at

a first stage to react the solution with the enzyme for a fixed period of time, and then adding TG to the reaction mixture for a reaction at a second stage.

[0027] The third process comprises adding TG to a solution containing milk or a milk protein for a reaction at the same time as a milk-clotting enzyme is added to the solution.

[0028] TG may be added for the reaction before the addition of the milk clotting enzyme. However, the above-mentioned three processes are preferable.

[0029] The conventional method of producing cheese can be applied except for the above-mentioned incorporation of the step of adding TG for the reaction and the order of that step.

[0030] TG which is used in the present invention may be derived from any source so far as it exhibits TG activity.

Examples of TG include TG derived from microorganisms belonging to the genus *Streptomyces* and the like [hereinafter abbreviated as "BTG"; refer to Japanese Laid-Open Patent Application (Kokai) No. 27,471/1989], TG derived from mammals such as guinea pigs (hereinafter abbreviated as "MTG"; refer to Japanese Patent 2nd. Publication (kokoku) No. 50,382/1989), TG derived from fish such as a cod and the like [Seki Nobuo et al., "Bulletin of the Japanese society of Scientific Fisheries", vol. 56, No. 1, p. 125 (1990)], and TG which is obtained through gene recombination [refer to Japanese Laid-Open Patent Application (Kokai) Nos. 300,889/1989, 199,883/1993 and 225,775/1994]. Of these, BTG is preferable because it acts in the absence of calcium and can be obtained in a large amount.

[0031] The concentration of TG added is between 0.1 U and 50 U, preferably between 0.5 U and 10 U per gram of a protein of a cheese material. When the concentration of TG is lower than 0.1 U, the effect expected by the use of TG is not obtained. When it is higher than 50 U, TG acts excessively. As a result, milk proteins are aggregated excessively, a gel structure of the curd is destroyed, the amount of the curd obtained is decreased, and the resulting cheese is crumbly, making it hard to obtain a block of cheese.

[0032] Further, the addition of TG higher than 50U leads to the undesirable effect that (1) the cheese is not smooth on the tongue, and (2) the cheese obtained as a final product does not have good taste. When cheese is produced according to the present invention, it is important to control the amount of TG used for obtaining the desired effects.

[0033] TG activity in the present invention is determined and defined as follows. That is, a reaction system containing benzyloxycarbonyl-L-glutamylglycine and hydroxylamine as substrates is reacted with TG in a tris buffer (pH 6.0) at a temperature of 37°C, and hydroxamic acid formed is transformed into an iron complex in the presence of trichloroacetic acid. Then, the absorbance of 525 nm is measured, and the amount of hydroxamic acid is calculated using a calibration curve. Thus, an amount of the enzyme by which 1  $\mu$  mole of hydroxamic acid for 1 minute is formed is defined as 1 unit (1 U) which is a unit of TG activity [refer to the description in the specification of Japanese Laid-Open Patent Application (Kokai) No. 27,471/1989]

[0034] The processes in which TG is added to cheese in the present invention will be described in detail below.

[0035] When cheese is produced in accordance with the present invention, it is important to incorporate the step of adding TG for the reaction. As mentioned above, there are the three processes. When the present invention is conducted, the most suitable process may be selected from these three processes depending on the kind of cheese to be produced, the limitation of the production line and the like.

[0036] In the first process, TG is added to a solution containing milk or a milk protein at a first stage, the mixture is heat-treated at a second stage, and a milk clotting enzyme is added to the reaction mixture at a third stage to react the reaction mixture with the milk clotting enzyme for a fixed period of time. According to this process, the heat treatment is conducted after the solution is reacted with TG. Therefore, the TG added is deactivated, and, advantageously, no TG activity is left in the final product. At this time, the conditions for the heat treatment are not particularly limited. The heat treatment is usually conducted at from 72 to 75°C for from 15 seconds to 2 minutes. It is considered that if TG activity remains in the final product in the case of the maturing of the cheese over a long period of time, it may be often that the properties of the cheese are changed during storage. However, when the process includes the step of deactivating TG through heating as mentioned above, such a change during storage can be avoided.

[0037] In the second process, a milk clotting enzyme is added to a solution containing milk or a milk protein at a first stage to react the solution with the enzyme for a fixed period of time, and then TG is added to the reaction mixture for a reaction at a second stage. The second process is, unlike the first process, characterized in that the solution containing milk or the milk protein is first reacted with the milk clotting enzyme, and the reaction mixture is then reacted with TG which is an enzyme to crosslink and polymerize the protein. In some kinds of cheese, this order sometimes exhibits the effect of the present invention remarkably. The process which is considered optimal may be selected from among these three processes.

[0038] In the third process, TG is added to a solution containing milk or a milk protein for a reaction at the same time a milk clotting enzyme is added to the solution. When the above-mentioned first and second processes cannot be adopted in view of the limited conditions for the production, cheese may be produced through the third process. This process in which TG is added simultaneously with the addition of the milk clotting enzyme is advantageous in that the change of the conventional production steps is minimized and the step of adding TG is incorporated therein.

[0039] In the second and third processes, TG activity is left in the curd formed. However, in a fresh-type cheese which

is stored for a short period of time, the change in the properties of cheese during storage is little. Even if the TG activity is left in the product which is distributed after the heat treatment of the final product, it poses no problem.

[0040] The means for adding TG is not particularly limited except that the step of adding TG for the reaction is incorporated according to any of the above-mentioned three processes. Examples of the milk clotting enzyme include animal rennets such as a calf rennet and a swine pepsin, plant rennets and microorganism rennets.

[0041] The animal rennets are preferable. Rennets which are produced through the genetic engineering are also available.

[0042] When the starting milk is reacted with TG according to the process of the present invention, various additives can be used in order to make TG exhibit the more desired effect. For example, calcium chloride may be added to expedite the formation of the curd in the renneting step.

[0043] In the present invention, cheese can be produced according to the conventional method (including a starting material) except that the step of adding TG for the reaction is incorporated according to any of the above-mentioned three processes.

[0044] When the solution is reacted with TG, a fixed reaction time and a fixed reaction temperature are needed. The usual production of cheese includes the renneting step and the heating step which is called the "cooking step". Accordingly, when the second or third process is employed, there is no need for employing a new step of reacting the solution with TG. If the enzyme is added before or during the renneting step or the cooking step, the effect of the present invention can be obtained satisfactorily during the renneting step or the cooking step.

[0045] Since the present invention provides the process for producing cheese, namely, natural cheese, a step of acidifying the solution containing milk or the milk protein with a lactic-acid bacillus starter is conducted before or simultaneously with the addition of the milk clotting enzyme. A mold starter is used in producing some kinds of cheese.

[0046] When the first process of the present invention is conducted, a suitable reaction temperature and a suitable reaction time are needed after the addition of TG. When the reaction is conducted, for example, at from 10 to 40°C for a reaction time of from 1 to 16 hours, the effect of the present invention is obtained sufficiently. When the temperature is lower than 10°C or higher than 40°C, a suitable effect can be obtained by appropriately controlling the reaction time. Accordingly, the TG reaction conditions are not particularly limited.

[0047] Thus, a cheese curd having excellent firmness and body and other properties can be obtained at a high yield by only incorporating the step of adding TG for the reaction into the conventional method of producing cheese without greatly changing the conventional starting materials, conventional additives and conventional steps which have been employed so far to produce cheese.

[0048] The cheese produced by the present invention is the same as the other kinds of cheese which are produced through traditional methods peculiar to them in terms of the taste and flavor, and it also has the same level of quality as the latter. The greatest advantage of the present invention is that cheese having the same quality can be produced in a large amount from the fixed amount of the starting milk.

[0049] In addition, it is also possible to produce cheese having a novel taste and a novel texture which have not been found in conventional cheeses.

[0050] Cheddar cheese which is produced by the process of the present invention markedly exhibits the effect of the present invention. Cheddar cheese is a so-called hard cheese (water content of less than approximately 40%) which is currently the cheese that is produced in the largest amount outside of Japan. This is used as a starting material for process cheese, and the mild taste thereof is agreeable to the Japanese people in general.

[0051] The present invention which increases the yield of the curd for hard cheese such as cheddar cheese, which gives curd having excellent firmness, body and texture, and which provides high-quality cheese is industrially quite useful.

[0052] Further, when so-called soft cheese is produced in accordance with the present invention, the yield of curd is increased and the effect of preventing serum separation (referred to as "syneresis" or "water separation") is also provided. Soft fresh cheese such as quark or cottage cheese is difficult to keep in that it causes water separation during storage. Japanese Laid-Open Patent Application (Kokai) No. 252,866/1993 describes a means for adding a stabilizer such that heat-sterilized fresh cheese does not cause aggregation and serum separation and has an excellent texture. The soft cheese which is produced by the process of the present invention is advantageous in that it does not cause serum separation during storage even if a stabilizer or the like is not added and it provides a smooth and comfortable texture.

#### Examples

[0053] The present invention will be illustrated specifically in referring to the following Examples.

Example 1 (Production of cheddar cheese)

[0054] Starting milk (11 kg; fat content = 3.40%) was sterilized, cooled, and then heated up to 31°C. Then, 2.25 g of a mixed lactic acid starter (*S. lactis*, *S. Cremoris*, made by Chris. Hansen's Laboratories) were added thereto, and the mixture was kept at 31°C for 60 minutes (step of fermenting lactic acid). When the step of fermenting lactic acid was conducted for 35 minutes, 0.72 ml of annatto food color were added. Five minutes later, 0.02% of calcium chloride were added. After the lactic acid fermentation step lasting 45 minutes, 2.25 ml of a calf rennet (single strength, made by Chris. Hansen's Laboratories) were added, and the mixture was allowed to stand for from 25 to 30 minutes to form a curd (renneting step). The formation of the curd was confirmed, and the curd was cut (cutting step). After the completion of the cutting step, the product was allowed to heal for 5 minutes. The curd was gently stirred for 10 minutes, and heating was then started (cooking step). In the cooking step, first the temperature was elevated from 31°C to 33°C, and the heating was conducted for 15 minutes, when TG was added. The amount of TG added was 10 U per gram of the protein (10 U/gp) in the starting milk. Subsequently, the temperature was elevated from 33°C to 35°C over a period of 15 minutes, and further from 35°C to 38°C over a period of 10 minutes. During the cooking step, the curd was slowly stirred so as not to crush the curd particles. Then, the stirring was continued at 38°C for 15 minutes, and the curd was allowed to stand for from 5 to 10 minutes. Then, the whey was drained.

[0055] After the whey was drained, the resulting curd was cut to into 6 inches wide, and cut pieces were overlaid one over the other. These cut pieces were kept at from 37°C to 38°C, and flipped every 15 minutes to prompt the drainage of the whey (cheddaring step). Thereafter, a step of milling the curd was conducted. The milled curd was gradually mixed with NaCl. At this time, NaCl was added for a total of three times such that the concentration of NaCl reached 4.5% of the curd. The curd was put in the hoop, pressed, and ripened to obtain a cheddar cheese product. A cheddar cheese product which was produced in the above-mentioned manner except that TG was not added was prepared as a control product.

[0056] After the process of press, the weight of the curd and the dry weight of the curd were measured and compared. An organoleptical properties of the cheddar cheese was estimated after it had ripened for 30 days. The results are shown in Table 1.

[0057] Thus, in the cheddar cheese which was produced with the addition of 10 U/gp of TG, the yield of the curd was increased by approximately 20%. The TG product had sufficient firmness and good body as hard cheese, and it was equal to the control product with respect to flavor, taste and appearance. Thus, the product was acceptable.

Table 1

Results of organoleptic evaluation		
	Control product (without addition of TG)	TG 10 U/gp
Weight of the curd (g)	878	1052
Dry weight of the curd (g)	518	616
Solids content of the curd (g)	59	59
Yield of the curd (%) (yield of the control curd is defined as 100%)	100	119
Condition of the block of cheese	excellent	excellent
Color	typical yellow of cheddar cheese	yellow approximately equal to that of the control product
Flavor, body and texture	suitable firmness, smooth texture	suitable firmness, no bitterness

Example 2

[0058] Cheddar cheese was produced in the same manner as in Example 1 except that TG was added in excessive amounts of 50 U/gp and 100 U/gp.

[0059] When 100 U/gp of TG were added, a curd was obtained in which the weight of the pressed curd was 918 g and the dry weight of the curd was 552 g after the press process. On the other hand, when 50 U/gp of TG were added, a curd was obtained in which the weight of the pressed curd was 1,233 g and the dry weight of the curd was 757 g after the press process. When 100 U/gp of TG were added, the yield of the curd was higher than that in the control product,

but it was lower than that given by the addition of 10 U/gp of TG and 50 U/gp of TG.

[0060] It proved to be important that TG is added at a concentration of within the optimum range in order to obtain the effect of an increase in the yield of the curd of hard cheese. Further, the results of the organoleptic evaluation revealed that the cheddar cheese obtained by the addition of 100 U/gp of TG was a little bit inferior to that obtained by the addition of 10 U/gp and 50 U/gp of TG with respect to the body, texture and condition of the block of cheese.

### Example 3 (Production of quark)

[0061] TG was added to 20 kg of starting milk (solids content of skim milk = 8.2%; fat content = 3.5%) at 25°C (The amount of TG is 5 unit per gram of the protein (5 U/gp) in the starting milk.) and the reaction was conducted at 25 °C for 2 hours. After the completion of the TG reaction, the reaction mixture was heated up to 75°C (TG deactivation), and cooled to 28°C. Then, 200 g of a mixed lactic acid starter and 0.01 g of a rennet were added, and the mixture was stirred.

[0062] The mixture was fermented to a pH of 4.7 at 28°C for approximately 5 hours to form a curd. The curd was packed into a bag for filtration, and the whey was drained through cooling at from 5 to 10°C to prepare quark. This quark was kneaded with the addition of 0.5% of NaCl to form a product.

[0063] As a control product, quark which was produced in the above-mentioned manner except that TG was not added was prepared. The results are shown in Table 2. By the way, quark is an unripened, soft-type fermented milk curd cheese which is produced in Germany, and elsewhere.

### Example 4 (Production of quark)

[0064] Twenty kilograms of starting milk (solids content of skim milk = 8.2%; fat content = 3.5%) were sterilized and cooled. Subsequently, the temperature was elevated to 27° C, and 200 g of a mixed lactic-acid bacillus starter, 0.01 g of a rennet and 25 ml of a calcium chloride aqueous solution were added. At the same time, TG was added in an amount of 1 unit per gram of milk protein (1 U/gp), and the fermentation was conducted at 27°C for 6 hours.

[0065] The curd formed was packed into a bag for filtration, and whey was drained through cooling at from 5 to 10°C to prepare quark. This quark was kneaded with the addition of 0.5% of NaCl to form a product.

[0066] As a control product, quark which was produced in the above-mentioned manner except that TG was not added was prepared. The weight, dry weight and protein amount of the curd in the quark in Examples 3 and 4 were measured and compared. Further, the organoleptical properties of the quark was estimated. The results are shown in Table 2 below.

Table 2

Results of organoleptic evaluation				
	Example 3 Control product	Example 3 TG 1 U/gp	Example 4 Control product	Example 4 TG 5 U/gp
Weight of the curd (kg)	3.02	3.62	3.43	3.88
Dry weight of the curd (kg)	0.69	0.69	0.74	0.76
Solids content of the curd (%)	22.8	19.1	21.6	19.6
Yield of the curd (%) (yield of the control product is defined as 100%)	100	120	100	113
Color	white	white	white	white
Serum separation (after 3 days of production)	+++	-	++	-

Table 2 (continued)

Results of organoleptic evaluation				
	Example 3 Control product	Example 3 TG 1 U/gp	Example 4 Control product	Example 4 TG 5 U/gp
Body and texture	suitable consistency, slightly dry and crumbling	suitable consistency, smooth texture	slightly dry and crumbling and rough texture	suitable consistency, good taste

[0067] Thus, when quark was produced with the addition of TG, a large amount of the curd could be obtained. The quark had a suitable consistency, creamy feeling and smooth texture, and the appearance thereof was excellent without causing serum separation. The quark obtained by the addition of TG was increased in the water content, but was not watery, and had a rather mild, palatable taste.

[0068] Also in Example 3 according to the process as mentioned in claim 1 and Example 4 according to the process as mentioned in claim 3, the organoleptically excellent curd could be obtained in a larger amount than the curd of the control product, though there was a slight difference between Examples 3 and 4 in the improvement of the yield of the curd.

#### Effects of the Invention

[0069] As mentioned above, the present invention can provide a cheese curd in a larger amount than that through the conventional method, by incorporating a very simple step of adding TG for a reaction in a fixed order into the process for producing so-called the conventional natural cheese including the step of adding milk clotting enzyme as a cheese starter. Thus, the starting milk can be used effectively.

[0070] Further, the obtained cheese has a quality which is acceptable to consumers without impairing the properties, such as flavor, taste and appearance peculiar to various kinds of cheese which are produced by the traditional methods.

#### Claims

1. A process for producing natural cheese, wherein a cheese curd is produced from a solution containing milk or a milk protein and the curd is separated from the whey,

said process comprising the steps of

- (1) adding between 0.1 U and 50 U transglutaminase per gram protein to the solution containing milk or the milk protein for a reaction at a first stage,
- (2) heat-treating the mixture at a second stage to de-activate the transglutaminase and
- (3) adding a milk clotting enzyme at a third stage to react the mixture with the milk clotting enzyme for a fixed period of time.

2. A process for producing natural cheese, wherein a cheese curd is produced from a solution containing milk or a milk protein and the curd is separated from the whey,

said process comprising the steps of

- (1) adding a milk clotting enzyme to a solution containing milk or a milk protein at a first stage to react the solution with the enzyme for a fixed period of time, and then
- (2) adding between 0.1 U and 50 U transglutaminase per gram protein to the mixture for a reaction at a second stage.

3. A process for producing natural cheese, wherein a cheese curd is produced from a solution containing milk or a milk protein and the curd is separated from the whey, which comprises adding between 0.1 U and 50 U transglutaminase per gram protein to the solution containing milk or the milk protein for a reaction and, at the same time, adding a milk clotting enzyme to the solution.

4. The process of any of the claims 1 to 3, wherein the solution containing milk or the milk protein is acidified using a lactic acid starter either before or simultaneously with the addition of the milk clotting enzyme.



## Patentansprüche

1. Verfahren zur Herstellung von natürlichem Käse, wobei Käsequark aus einer Lösung hergestellt wird, die Milch oder ein Milchprotein enthält, und der Quark von der Molke abgetrennt wird, wobei das Verfahren folgende Schritte umfaßt:

(1) Zugabe zwischen 0,1 U und 50 U Transglutaminase pro Gramm Protein zu der Lösung, die Milch oder das Milchprotein enthält, für eine Reaktion in einer ersten Stufe,  
 (2) Hitzebehandlung des Gemisches in einer zweiten Stufe zur Desaktivierung der Transglutaminase und  
 (3) Zugabe eines Milchgerinnungsenzyms in einer dritten Stufe, wobei das Gemisch mit dem Milchgerinnungsenzym während eines festgesetzten Zeitraums umgesetzt wird.

2. Verfahren zur Herstellung von natürlichem Käse, wobei Käsequark aus einer Lösung hergestellt wird, die Milch oder ein Milchprotein enthält, und der Quark von der Molke abgetrennt wird, wobei das Verfahren folgende Schritte umfaßt:

(1) Zugabe eines Milchgerinnungsenzyms zu einer Lösung, die Milch oder ein Milchprotein enthält, in einer ersten Stufe, um die Lösung mit dem Enzym während eines festgesetzten Zeitraums umzusetzen, und dann  
 (2) Zugabe zwischen 0,1 U und 50 U Transglutaminase pro Gramm Protein zu dem Gemisch für eine Reaktion in einer zweiten Stufe.

3. Verfahren zur Herstellung von natürlichem Käse, wobei Käsequark aus einer Lösung hergestellt wird, die Milch oder ein Milchprotein enthält, und der Quark von der Molke abgetrennt wird, welches das Zugabe zwischen 0,1 U und 50 U Transglutaminase pro Gramm Protein zu der Lösung, die Milch oder das Milchprotein enthält, für eine Reaktion und das gleichzeitige Zugabe eines Milchgerinnungsenzyms zu der Lösung umfaßt.

4. Verfahren gemäß einen der Ansprüche 1 bis 3, wobei die Lösung, die Milch oder das Milchprotein enthält, unter Verwendung eines Milchsäurestarters entweder vor der oder gleichzeitig mit der Zugabe des Milchgerinnungsenzyms angesäuert wird.

## Revendications

1. Procédé de production de fromage naturel, dans lequel un caillé de fromage est produit à partir d'une solution contenant du lait ou une protéine de lait et le caillé est séparé du petit-lait,

ledit procédé comprenant les étapes

(1) de l'addition d'une quantité de transglutaminase entre 0,1 U et 50 U par gramme de protéine à la solution contenant du lait ou une protéine de lait pour qu'une réaction ait lieu dans une première étape,  
 (2) du traitement thermique du mélange dans une seconde étape pour désactiver la transglutaminase et  
 (3) de l'addition d'une enzyme provoquant le caillage du lait dans une troisième étape pour faire réagir le mélange avec l'enzyme provoquant le caillage du lait pendant une durée déterminée.

2. Procédé de production de fromage naturel, dans lequel le caillé de fromage est produit à partir d'une solution contenant du lait ou une protéine de lait et le caillé est séparé du petit-lait,

ledit procédé comprenant les étapes

(1) de l'addition d'une enzyme provoquant le caillage du lait à une solution contenant du lait ou une protéine de lait dans une première étape pour qu'une réaction ait lieu entre la solution et l'enzyme pendant une durée déterminée et ensuite,  
 (2) de l'addition d'une quantité de transglutaminase entre 0,1 U et 50 U par gramme de protéine au mélange pour qu'une réaction ait lieu dans une deuxième étape.

3. Procédé de production de fromage naturel, dans lequel le caillé de fromage est produit à partir d'une solution contenant du lait ou une protéine de lait et le caillé est séparé du petit-lait, comprenant l'addition d'une quantité de transglutaminase entre 0,1 U et 50 U par gramme de protéine à une solution contenant du lait ou une protéine de lait pour qu'une réaction ait lieu et, en même temps, l'addition d'une enzyme provoquant le caillage du lait dans la solution.

1. Procédé selon une quelconque des revendications 1 à 3, dans lequel la solution contenant du lait ou une protéine de lait est acidifiée au moyen d'un levain lactique soit avant soit en même temps que l'addition de l'enzyme provoquant le caillage du lait.

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